CHEMICAL STUDIES ON THE SOLUBLE STARCH FRACTION IN BREAD

by

HORST BECK

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INTRODUCTION

Amylases are enzymes which catalyze starch hydrolysis, converting the starch into dextrins and maltose (43). There are two main types of amylases, alpha-amylase and beta-amylase. The beta-amylase splits maltose units from the non-reducing end of the starch chains and is called also a saccharogenic or saccharifying enzyme. The alpha-amylase splits the chain at almost any α-1,4 glucosidic linkage (30). Of the reducing material formed, a considerable part consists of dextrins. Therefore, the alpha-amylase is known also as dextrinogenic amylase. The alpha- and beta-amylase complement each other's action. Following amylolysis there always remain low molecular weight dextrins which include α-1,6 glucosidic linkages. These are derived from the amylopectin. The amylose fraction may be completely converted to maltose by the action of beta-amylase (29).

Pazur and Sandstedt (41) studied the reducing groups produced by the action of malt enzymes on starch and starch-oligosaccharides, at 30° C. They used paper chromatography to resolve and identify the different sugars. The action of alpha-amylase on starch resulted in the production of several short chain saccharides, including glucose, maltose, amylotriose, amylotetraose, and amylopentaose. Maltose was the only low molecular weight product obtained by the action of beta amylase on the starch. Alpha-amylase hydrolyzed amylotriose slowly to glucose and maltose, but beta-amylase did not hydrolyze it. Glucose, maltose and amylotriose were obtained by the hydrolysis of

amylotetraose with alpha-amylase, but only maltose resulted when the same was hydrolyzed with beta-amylase.

Sandstedt et al (45) have shown that the undamaged starch granules of wheat flour are not attacked by the beta-amylase, but the damaged granules are readily available to its action. Beta-amylase is present in sufficient amount in normal wheat flour, but alpha-amylase has to be supplied (17) in the form of malted cereal or other purified forms.

Malt syrup has long been used in bakery products. Its dough improving properties were recognized in the 1890's (43). At the present time, malted flours generally are used to supplement alpha-amylase. Most of this malt is obtained from barley or wheat and, to a much smaller extent, from corn.

In recent years, several studies have been made on the use of malt supplements from sources other than cereals for bread-making purposes. Miller and Johnson (37) compared alpha-amylase supplements from fungi and wheat malt, at different concentrations and in straight and sponge doughs. Conn et al (11), studying alpha-amylase preparations from bacterial and fungal sources as compared with malted wheat flour, concluded that fungal preparations may be used for commercial alpha-amylase supplementation if the proteinase content is not too high. The bacterial preparations gave sticky and gummy bread crumb. Johnson and Miller (26, 27) have shown the advantages of fungal alpha-amylase supplements.

Schultz et al (49) studied the retardation of bread crumb staling in commercial bread by bacterial alpha-amylase at vari-

ous concentrations. Jackel et al (24) studied the distribution and behavior of the soluble and insoluble starch fractions of bread crumb, with relation to their susceptibility to hydrolysis by beta-amylase. They compared the results obtained in white bread baked with and without bacterial alpha-amylase supplementation.

Miller et al (38) compared cereal, fungal, and bacterial amylase preparations as supplements for breadmaking. They found that the firmness of the bread crumb, as well as the amount and the characteristics of the dextrin extracted from bread stored for 90 hours, seemed to be related to the thermostability of the enzymes used.

Depending upon their source, the amylases exhibit differences in certain properties, like optimum pH, optimum temperatures, and thermostability (43). The bacterial alpha-amylase is the most thermostable, fungal alpha-amylase is the least thermostable, while the thermostability of the cereal alpha-amylase is intermediate (38). The presence of starch tended to protect the bacterial amylase against inactivation by heat, a characteristic not shown by the other enzymes. An increase in concentration of the enzymes lowered the rate of firming of the bread crumb, and had the tendency of producing more complex dextrins. The amount of extractable dextrin in bread increased with all amylases, but, with bacterial amylase, this increase was much greater than with fungal or cereal amylase. With the aging of the bread the average molecular weight of the dextrins decreased in the soluble fraction, due to the crystallization or insolu-

bilization of the larger dextrins.

Various methods for determining breakdown products of starch are available. A few were selected for the purpose of this study:

Periodic acid and periodates have been used by many investigators for the determination of average chain lengths of carbohydrates (1, 7, 10, 12, 20, 25, 33, 35, 39, 44, 47). The conditions of pH, temperature, and time period at which the oxidation was carried out were not the same for all studies. The method used in the present work was based on that of Abdel-Akher and Smith (1).

The ferricyanide method for measurement of reducing groups (2, 3) was used by Miller et al (38) for the estimation of the average chain length of dextrins. It has the advantage of being rapid. The colorimetric ferricyanide method, like the previous method, is based on the oxidation of the aldehydic group by ferricyanide. The addition of ferric sulfate produces Prussian blue, which is stabilized by gum ghatti. The color intensity of the Prussian blue is measured with a spectrophotometer at 520 mμ. It has been used by Nussenbaum and Hassid (40) for the determination of average chain length, in comparison with osmotic pressure measurements.

There are several other workable methods, not used in the present study. These include: a great number of other reduction methods, among which the copper reduction methods are the most important; the methylation method (4, 6, 22) which is the classical procedure for the determination of structure and chain

length, but which is very elaborate and time-consuming; and the osmotic pressure determination of dilute solutions (16, 19, 21, 42).

Many methods have been used for the determination of sugars (8), using physical, chemical, and biological means. Most of these methods fail to differentiate among the different types of sugar present in a mixture. Several methods have been developed for the analysis of sugar mixtures. Most of them are elaborate. Paper partition chromatography supplies an ideal method for the separation of the sugars present in material such as flour and bread extracts (18, 31). Once separated, several analytical methods may be used for the quantitative determination of each sugar (2, 3, 8, 14, 32, 36, 50). Basically, chromatography is a fractionation procedure (51). It depends on the differential migration of solutes through a stationary phase. In paper partition chromatography, the stationary phase is generally water supported by a sheet, strip, or circle of filter paper. The moving solvent usually has two or more components. The solvent carries the solutes at different speeds through the paper, depending on the partition coefficient of each solute. The partition coefficient is the ratio between the concentration of the solute in the non-mobile phase and the concentration of the same in the mobile phase, at equilibrium (5).

The objective of the present study was to characterize the carbohydrate material altered during baking by the different types and concentrations of alpha-amylase supplements and to relate these data to the characteristics of the bread crumb.

The characteristics studied included concentration of maltose and dextrose, and quantity and chain length of the soluble dextrins.

MATERIALS AND METHODS

Baking of Bread

An unmalted commercial hard red winter patent flour with 0.41 per cent ash and a protein content of 11.8 per cent (14 per cent moisture basis) was used for all experiments.

The enzyme supplements included a commercial malted wheat flour and two commercial enzyme concentrates, one of which was of fungal (Diastase-33[1]) and the other of bacterial (Rhozyme DX[1]) origin. The alpha-amylase activities of these preparations were 75, 5335, and 253 SKB units (46) per gram, respectively.

The baking experiments were carried out on a laboratory scale using a 70 per cent sponge-dough procedure. The enzyme supplementation was made in the sponge.

The following formula was used:

Ingredients	Percentage	Sponge	Dough
		gm	gm
Flour	100.0	490.0	210.0
Yeast	2.0	14.0	--
Arkady	0.25	1.75	--
Malt supplement	variable	variable	--
Sugar	5.0	--	35.0
Salt	2.0	--	14.0

[1] Rohm and Haas Co., Philadelphia, Pennsylvania.

Ingredients	Percentage	Sponge gm	Dough gm
Dry skim milk solids	4.0	--	28.0
Shortening	3.0	--	21.0
Water	70.0	343.0	147.0

A total of ten variations in enzyme supplements was included. These were as follows:

1. Control.
2. Malted wheat flour--140 alpha-amylase units.
3. " " " 560 " " "
4. " " " 1120 " " "
5. Diastase-33 140 " " "
6. " " 560 " " "
7. " " 1120 " " "
8. Rhozyme (DX) 7 " " "
9. " " 35 " " "
10. " " 140 " " "

The sponges were mixed for two minutes in a Hobart A-200 mixer. They were set at 81-82° F. and fermented for three hours at 86-88° F. and 90 per cent relative humidity.

The doughs were remixed for three minutes and the dough temperature controlled at 84-86° F. The doughs were given a 30-minute floor time, scaled to 18 oz., and given 20 minutes' rest before moulding. The doughs were proofed at 92-94° F. and 98 per cent relative humidity for 55 minutes and baked for 28 minutes at 425° F. in a revolving reel type oven. Loaf volumes and weights were determined immediately after coming from the oven. Grain and texture scores were made one hour after baking. After scoring, the sugars and the soluble starch fractions were extracted from the crumb.

Preparation of the Crumb Extract

The sugars from 100 gm of bread crumb were extracted by refluxing for two and a half hours with 320 ml of 95 per cent ethyl alcohol. This would give a final extract of 84 per cent alcohol due to the water contained in the crumb. The suspension was centrifuged to separate the extract from the crumb. Most of the alcohol was removed from this extract by distillation under low pressure. The concentrate was centrifuged at 1400 r.p.m. for five minutes, and made up to 50 ml volume.

The solution was then de-ionized (52) by adding 2 gm each of Dowex 50[1] and Duolite A4[2], and letting it stand for 10 minutes. It was then filtered and stored in the refrigerator until the chromatographic analysis could be performed.

The residue of the alcohol extraction, remaining in the centrifuge-tubes, was washed into a one-liter Erlenmeyer flask with 500 ml water and extracted for two hours at 30° C. It was then centrifuged at 1400 r.p.m. during five minutes, the aqueous solution decanted, placed in test tubes, and rapidly frozen at -10° C. for storage. Aliquots of this solution were used for the determination of the amount of soluble dextrins and for the determination of their average chain length by the various methods.

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[1] Dow Chemical Co., Midland, Michigan.
[2] Chemical Process Co., San Francisco, California.

Determination of the Soluble Starch Fraction in Bread

To determine the amount of soluble dextrin, the contents of the test tube was thawed rapidly in a boiling water bath. After cooling, 20 ml aliquots were taken to which 100 ml of 95 per cent ethyl alcohol was added. The flasks were stored in the refrigerator overnight and the contents filtered through a tared Gooch crucible. The precipitate was washed with 10 ml of 80 per cent ethyl alcohol, dried at 130° C. for two hours, and weighed (6).

Determination of the Average Chain Length of the Dextrins

The average chain length of soluble dextrins present in the crumb of the breads obtained with the various enzyme supplements was determined in aliquots of the above described aqueous dextrin solutions. Three different methods were used for these determinations:

1. Periodate method (1).
2. Ferricyanide method (2).
3. Colorimetric ferricyanide method (40).

Periodate Method. This method was carried out as follows: To a 20 ml aliquot of the aqueous dextrin solution was added 20 ml of 0.25 M sodium metaperiodate. This solution was diluted to 50 ml with water and stored at 5-6° C. in the dark. Concurrently, a "blank" was analyzed under the same conditions. Aliquots

(5 ml) were removed at suitable intervals. To free the solutions from the remaining metaperiodate, 0.5 ml of ethylene glycol was added, and the reaction allowed to proceed in dim light during 10 minutes. An excess of potassium iodide (5 ml of 10 per cent solution) was added, followed by a slight excess of 0.01 N sodium thiosulfate. The excess of sodium thiosulfate was then back-titrated with 0.01 N iodine, using starch as indicator. A micro-burette was used in this titration. Along with the samples and the blank, a standard solution of glucose was subjected to the same treatment.

Ferricyanide Method. Analyses were performed both before and after hydrolysis of the dextrin solutions with 1 N sulfuric acid. In this method, a 5 ml aliquot of the dextrin solution was pipetted into a test tube of about 50 ml capacity. Ten ml of 0.1 N alkaline ferricyanide solution was added, mixed by swirling, and the test tube immersed in a vigorously boiling water bath for exactly 20 minutes, maintaining the liquid in the tube 3-4 cm below the surface of the boiling water. The test tube and contents was cooled immediately under running water, and transferred to a 125 ml Erlenmeyer flask. The test tube was rinsed out twice with a total of 25 ml of acetic acid-salt solution, and the rinsings added to the solution in the Erlenmeyer flask. One ml of soluble starch-potassium iodide solution was added to the flask and the contents titrated with 0.1 N thiosulfate to the complete disappearance of the blue color. Along with the samples, blanks and standard solutions of glucose were submitted to the same treatment.

Colorimetric Ferricyanide Method. In this method, 1 or 2 ml aliquots of solution containing from 5 to 25 mg of dextrins were pipetted into test tubes. One-half ml of an 0.8 per cent solution of potassium ferricyanide and water, if necessary, was added to each tube to bring the total volume to 2.5 ml. One-half ml of carbonate-cyanide solution was added, the contents mixed, and immediately heated in a boiling water bath for exactly eight minutes. The tubes were then cooled, 5 ml of ferric sulfate-gum ghatti solution added, the contents mixed, and allowed to stand for five minutes. Two ml of water was then added to dilute the volume to 10 ml. Blanks and standard solutions of glucose and maltose were prepared in the same way, and the optical density measured at 520 mμ in a Coleman Universal spectrophotometer. The standard curves are presented in the Appendix, Fig. 3.

The chain lengths of the dextrins was calculated from the reducing values before and after hydrolysis with 1 N sulfuric acid in terms of anhydroglucose residues.

Determination of Reducing Matter in Bread Crumb Extracts

The possible presence of reducing matter other than reducing carbohydrates in the bread extract required that a separate determination of reducing matter be made. For this purpose the iodometric method of Freilich (15) was used. In this method, the reducing matter in 5 ml of bread extract was allowed to react with 10 ml of 0.01 N iodine solution containing 0.24 gm of

potassium iodide. The amount of iodine reacting was determined by immediate back-titration with 0.01 N sodium thiosulfate.

Estimation of Maltose and Glucose in Bread Crumb

Maltose and glucose in the bread crumb were estimated by chromatographic analysis. The descending development was used to separate the mixture of sugars (34). A constant volume (4.8 µl) of sugar solution was spotted with a micro pipette on 4" x 22" Whatman No. 1 filter paper strips. The amount of sugar on each spot varied from 150 to 280 µg. After the spots had dried, the strips were suspended from glass solvent troughs placed in a tall chromatographic jar with the bottom covered by developing solvent. The glass plate used to cover the jar had holes for the introduction of the developing solvent. The developing solvent was a mixture of 1-propanol, ethanol, and water, in the proportion 7:1:2 (18). The chromatograms were developed for 40 hours.

On each paper strip, four spots of sugar were placed three-fourths of an inch apart. Three of the spots were from bread extracts and the other from the known sugar composite. After the strips had been developed and dried, they were cut lengthways in the middle. The half containing the known composite was sprayed with aniline-diphenylamine (9) for identification.

The position of the sugars on the paper strip having been determined, the quantitative determination of the same was made by the method established by Dubois et al (14). The other half of each paper strip was cut into pieces separating the maltose

and dextrose identified by the spray reagent. These pieces were then cut into two parts to separate the sugars coming from the two spots. Each of these duplicates was then extracted with 25 ml of water, and the extract filtered through glasswool. Two 5 ml aliquots were taken, mixed with 0.3 ml of 80 per cent aqueous phenol and 12.5 ml of concentrated sulfuric acid. After letting the color develop for at least 30 minutes, the optical density was determined at 490 mμ in a Coleman Universal spectrophotometer. The optical density was referred to a standard curve established in a similar way from known concentrations of the sugars. The standard curves for maltose and glucose are presented in the Appendix, Fig. 4.

RESULTS

Increasing amounts of each of the different types of alpha-amylase tended to increase the loaf volume of the bread. This increase was higher with cereal and fungal than with bacterial alpha-amylase. Grain and texture scores for each of the alpha-amylase types increased with the lower levels and decreased with higher amounts of enzyme, as shown in Table 1. The greater efficiency of the bacterial alpha-amylase caused by its thermostability is evident from these data.

Table 1. Effect of alpha-amylase type and concentration on the characteristics of bread.

Enzyme	Alpha-amylase SKB units (46)	Loaf volume cc	Crumb Scores	
			Grain %	Texture %
Control	None	2450	75	75
Malted wheat flour	140	2787	90	90
Malted wheat flour	560	3000	85	90
Malted wheat flour	1120	2862	80	85
Control	None	2450	75	75
Fungal	140	2750	95	95
Fungal	560	2900	85	85
Fungal	1120	2950	80	80
Control	None	2450	75	75
Bacterial	7	2600	90	90
Bacterial	35	2600	90	80
Bacterial	140	2637	75	60

The amount of soluble dextrins extracted from the bread crumb increased with increasing amounts of alpha-amylase. This increase was small with the fungal, more pronounced with the cereal, and much greater with the bacterial alpha-amylase. The average chain length of the dextrins extracted also increased with the increased amount of alpha-amylase. This increase in average chain length showed the same trend as the increase in amount of dextrins extracted, being small with fungal, medium with cereal, and large with bacterial alpha-amylase supplementation. The ferricyanide and the colorimetric ferricyanide method gave comparable results for the average chain length, while the periodate method gave results which were considerably higher than those obtained by the other methods (Table 2).

Table 2. Effect of alpha-amylase type and concentration on the quantity of soluble dextrins and their average chain lengths.

Enzyme	Alpha-amylase SKB units (46)	Soluble dextrins %	Average chain length in glucose units: Perio-date (1)	Ferri-cyanide (2)	Color-imetric (40)
Control	None	1.5	7	1.4	1.5
MWF[1]	140	2.2	10	1.9	2.3
MWF	560	3.1	12	1.9	3.3
MWF	1120	3.7	13	2.2	3.1
Control	None	1.5	7	1.4	1.5
Fungal	140	1.9	9	1.4	1.6
Fungal	560	2.1	10	1.6	1.6
Fungal	1120	1.9	9	1.6	1.9
Control	None	1.5	7	1.4	1.5
Bacterial	7	2.8	13	2.2	3.1
Bacterial	35	5.7	29	4.6	6.7
Bacterial	140	10.6	52	6.2	7.1

[1] Malted wheat flour.

Glucose and maltose concentration may be expected to vary with different alpha-amylase supplements since these sugars result from amylolysis. The concentration of these sugars increased with increasing alpha-amylase. The increase of glucose was about the same when the cereal and fungal supplements were employed. The increase with the same concentrations of bacterial enzyme was much more pronounced. The increase in maltose was less pronounced with fungal than with bacterial or cereal alpha-amylase supplements. This is shown in Plate I and by the data included in the Appendix (Table 3).

EXPLANATION OF PLATE I

Fig. 1. The relationship between glucose in bread crumb and alpha-amylase concentration.

Fig. 2. The relationship between maltose in bread crumb and alpha-amylase concentration.

PLATE I

Fig. 1

Fig. 2

DISCUSSION

During the fermentation of the bread dough, the alpha- and beta-amylases hydrolyze the small amount of starch made available for enzyme attack by damaging of the starch granules during the milling of the wheat. The bulk of the starch is not available for amylase attack during the fermentation. Since beta-amylase is usually present in excess (17), the quantity of starch hydrolyzed during fermentation depends primarily on the alpha-amylase present.

As baking of the bread proceeds, the increase in dough temperature brings about gelatinization of the starch and inactivation of the beta-amylase. The swollen starch is rapidly attacked by the alpha-amylase. At the same time, the heat tends to inactivate the enzymes. At the beginning, the increase in the rate of amylase activity is faster than the heat inactivation of the amylases. Therefore, at this stage of the breadbaking process, a considerable portion of the starch may undergo amylolysis. The quantity and also the source of the alpha-amylase influences the extent of starch hydrolysis during baking (38). The differential activity of the various alpha-amylases primarily is due to the difference in their thermostability. The bacterial alpha-amylase is more thermostable than cereal or fungal alpha-amylase. Bacterial amylase, therefore, acts for a longer time on the gelatinized starch during the breadbaking process. Therefore, it was to be expected that the bread sup-

plemented with bacterial would show a more extensive starch hydrolysis than that containing cereal amylase. Bread supplemented with fungal alpha-amylase would be expected to show a still smaller extent of starch hydrolysis. The least amount of hydrolysis was expected in the control which did not have any alpha-amylase added. These expectations are corroborated by data obtained in the present study (Table 2). The amount of soluble dextrins extracted from the bread crumb increased with the increase in quantity of alpha-amylase supplement. The fungal alpha-amylase increased the soluble dextrins very little because it was inactivated before much of the starch became gelatinized and available to enzyme attack. The amount of soluble dextrins was increased significantly by the bacterial alpha-amylase which which was active for a relatively long period of time after the starch became gelatinized. The cereal alpha-amylase, as expected, caused a medium increase in the amount of soluble dextrins.

In addition to the increase in amount of soluble dextrins with increasing alpha-amylase, the average chain length also increased (Table 2). This is understandable because the alpha-amylase, in contrast to the beta-amylase, splits the starch molecule in several relatively large fragments. Part of the dextrins formed are water soluble and can be extracted from the bread crumb. Extensive modification of the starch granules by enzyme action causes the average chain length of water soluble dextrins to be greater. The data in Table 2 were obtained in replicate experiments.

In the present data, the average chain length of the dextrins was not as long as that found by Miller et al (38). It was suspected that this might be due to the differences in the methods employed to extract the soluble dextrins or to the presence of non-carbohydrate reducing matter. If the water extract of dextrins contained a relatively large amount of reducing compounds other than reducing carbohydrates, the initial reducing power would be high and thus the calculated average chain length would be low. A measurement of the reducing matter present in the water extracts of the dextrins by an iodine titration method (15), however, showed that the amount of reducing matter was negligible.

The high initial reducing power of the extracts may be due to the incomplete removal of reducing sugars from the bread crumb by the alcohol extraction procedure. If this were true, the calculated average chain lengths would be low. This, coupled with the mild extraction procedure used to obtain the water soluble dextrins compared to the drastic procedure used by Miller et al (38), would account for the lower calculated average chain lengths in this study. Nevertheless, the trends exhibited by the present data confirm those of Miller et al (38).

It has been shown that the action of beta-amylase on starch produces only maltose, but hydrolysis with alpha-amylase produces glucose, maltose, and higher molecular weight sugars (41). It also has been shown that there is a linear relationship between sugar added to the dough and the residual sugar in the crumb (18). Similarly, a relationship might be expected between

the sugar produced during fermentation and baking, and the residual sugar of the bread crumb. Figure 1 indicates that an increase in the glucose content of the crumb was obtained with increased alpha-amylase. The increase in maltose was less with the fungal supplement because of the heat inactivation of the alpha-amylase early in the baking process.

The alpha-amylases modify the gel properties of the starch made available by the gelatinization, thus increasing the gas retention. The increased gas retention tended to increase the loaf volumes (Table 1). This modification varies with type and concentration of the amylase.

Modification of the grain and texture of the bread apparently is associated with the changes in the starch as a result of amylolysis by alpha-amylase. A small extent of starch modification would appear to change the gel structure sufficiently to permit its flow with greater ease. This presumably would cause the grain to be fine and the texture soft and resilient. More extensive modification would result in an open grain and excessively soft texture that lacks body. If amylolysis is carried still farther, the starch gel will not assume a rigid state. Much of the starch then would be in a "soluble state," and be subject to extraction by water. The bread crumb might be sticky if the amylolysis is extensive. The quantity of soluble dextrins in such bread crumb would be large.

Bacterial amylase tended to produce a somewhat sticky and gummy bread crumb and shows little promise as a diastatic supplement. This might be attributed to the severe modification of

the starch due to the prolonged action of the highly thermostable bacterial alpha-amylase.

CONCLUSIONS

The following conclusions may be drawn from the present study:

1. The bread loaf volume increased more with cereal and fungal than with bacterial alpha-amylase.

2. Grain and texture of the bread improved up to a maximum for each of the alpha-amylase types and then deteriorated with further increases in the alpha-amylase concentration. Effect of the alpha-amylase on the bread quality varied according to the source of the enzyme.

3. Bacterial alpha-amylase supplement tended to produce a sticky and gummy bread crumb.

4. Increased solubilization of the starch was observed with increasing amounts of each of the alpha-amylase types.

5. The average chain length of the dextrins extracted increased with increasing amounts of alpha-amylase.

6. The increase in average chain length of the dextrins was small with fungal, medium with cereal, and large with bacterial alpha-amylase supplementation.

7. The residual content of glucose in the bread crumb increased to a greater extent with bacterial than it did with cereal or fungal alpha-amylase.

8. The residual content of maltose in the bread crumb was

less with fungal than it was with bacterial or cereal alpha-amylase.

9. The differences in effect produced by the three types of alpha-amylase primarily are due to the differences in their thermostability.

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REFERENCES

(1) Abdel-Akher, M., and F. Smith.
The repeating unit of glycogen. Jour. Amer. Chem. Soc. 73:994-996. 1951.

(2) American Association of Cereal Chemists.
Cereal laboratory methods, 5th ed., American Association of Cereal Chemists. St. Paul, Minnesota. 341 p. 1947.

(3) Association of Official Agricultural Chemists.
Official Methods of Analysis of the Association Official Agricultural Chemists. Washington, D. C. Assoc. Official Agr. Chemists. 910 p. 1950.

(4) Barker, S. A., E. J. Bourne, and I. A. Wilkinson.
The enzymic synthesis and degradation of starch. Part IX. Methylation and end-group assay of some synthetic polysaccharides. Jour. Chem. Soc. (London). 3027-3030. 1950.

(5) Block, Richard L.
Paper Chromatography-A Laboratory Manual. New York: Academic Press. 195 p. 1952.

(6) Bourne, E. J., K. H. Fantes, and Stanley Peat.
A modified method for the end-group assay of amylose and other long-chain starch fractions. Jour. Chem. Soc. (London). 1109-1113. 1949.

(7) Brown, F., Sonia Dunstan, T. G. Hall, E. G. Hirst, and J. K. N. Jones.
Application of new methods of end-group determination to structural problems in the polysaccharides. Nature. 156:785-786. 1945.

(8) Browne, Charles A., and F. W. Zerban.
Physical and chemical methods of sugar analysis. New York: John Wiley & Sons. 1353 p. 1941.

(9) Buchan, J. L., and R. I. Savage.
Paper chromatography of some starch conversion products. Analyst. 77:401-406. 1952.

(10) Caldwell, C. G., and R. M. Hixon.
A study of the periodic acid oxidation of starches and dextrins as a means of determining molecular size. Jour. Biol. Chem. 125:595-606. 1938.

(11) Conn, J. F., John A. Johnson, and Byron S. Miller.
An investigation of commercial fungal and bacterial alpha-amylase preparations in baking. Cereal Chem. 27:191-205. 1950.

(12) Courteouis, Jean, and Marcel Ramet.
Analytical study of the oxidation of lactose and maltose by periodic acid. Bull. Soc. Chim. Biol. 29:240-248. 1947.

(13) Dubois, Michel.
Water-soluble carbohydrates of the wheat germ. Master Thesis, University of Minnesota, January 1951.

(14) Dubois, M., K. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.
A colorimetric method for the determination of sugars. Nature. 168:167. 1951.

(15) Freilich, J.
A method for the determination of reducing matter in flour. Cereal Chem. 18:129-137. 1941.

(16) Fuoss, Raymond M., and Darwin J. Mead.
Osmotic pressures of polyvinyl chloride solutions by a dynamic method. Jour. Phys. Chem. 47:59-70. 1943.

(17) Geddes, W. F.
The amylases of wheat and their significance in milling and baking technology. In Advances in Enzymology. (F. F. Nord, ed.) New York: Interscience. 6:415-468. 1946.

(18) Griffith, Thomas, and John A. Johnson.
Chromatographic analysis of sugars in bread. Cereal Chem. 31:130-134. 1954.

(19) Guggenheim, E. A., and M. L. McGlashan.
The determination of molecular weights of macromolecules from measurements of osmotic pressure. Trans. Faraday Soc. 48:206-211. 1952.

(20) Halsall, T. G., E. L. Hirst, and J. K. N. Jones.
Potassium periodate oxidation of glycogens. Jour. Chem. Soc. (London). 1399-1400. 1947.

(21) Hitchcock, David I.
Osmotic pressure and molecular weight. Jour. Chem. Education. 28:478-479. 1951.

(22) Hodge, J. E.,S.A.Karjala, and G. E. Hilbert.
Methylation and ethylation of corn starch, amylose and amylopectin in liquid ammonia. Jour. Amer. Chem. Soc. 73:3312-3316. 1951.

(23) Illingworth, Barbara, Joseph Larner, and Gerty T. Cori.
Structure of glycogens and amylopectins. I. Enzymatic determination of chain length. Jour. Biol. Chem. 199: 631-640. 1952.

(24) Jackel, S. S., W. E. Schaeder, and A. S. Schultz.
Susceptibility to hydrolysis by beta-amylase of the soluble and insoluble fractions of the crumb of conventional and bacterial alpha-amylase supplemented white bread. Cereal Chem. 30:190-201. 1953.

(25) Jeanes, Allene, and C. A. Wilham.
Periodate oxidation of dextran. Jour. Amer. Chem. Soc. 72: 2655-2657. 1950.

(26) Johnson, John A., and Byron S. Miller.
Fungal enzymes help you make better bread. I. Greater loaf volume, improved crumb characteristics are rewards, since these new supplements enable control of enzymic activity and dough consistency. Food. Ind. 80-83. March, 1951.

(27) Johnson, John A., and Byron S. Miller
Fungal enzymes help you make better bread. II. Today, mold supplements are available with any desired ratio of proteinase to alpha-amylase- giving bakers new control over doughs prior to make-up. Food. Eng. 161-163, 165, 167. April, 1951.

(28) Kerr, Ralph W.
Chemistry and Industry of Starch. New York:Academic Press. 719 p. 1950.

(29) Kneen, Eric.
Amylases in germinated cereals. Cereal Chem. 21:304-314. 1944.

(30) Kneen, Eric, and R. M. Sandstedt.
Application of the amylases in milling and baking technology. In Enzymes and Their Role in Wheat Technology. (J. A. Anderson, ed.) New York:Interscience. 89-126. 1946.

(31) Koch, R. B., W. F. Geddes, and F. Smith.
The carbohydrates of Gramineae. I. The sugars of the flour of wheat (Triticum Vulgare). Cereal Chem. 28: 424-430. 1951.

(32) Koehler, Leonare H.
Differentiation of carbohydrates by anthrone reaction rate and color intensity. Anal. Chem. 24:1576-1579. 1952.

(33) Laskowski, Leona, and Robert E. Burk.
The probable length of hydrocarbon chains. Jour. Chem. Phys. 7: 465-469. 1939.

(34) Lederer, Edgar, a nd Michael Lederer.
Chromatography - A review of principals and applications. New York:Elsevier. 460 p. 1953.

(35) Lindstedt, Gosta.
Periodate oxidation of sugars in neutral phosphate buffer. Nature. 156:448-449. 1945.

(36) McFarren, E. F., K. Brand, and H. R. Rulkowski.
Quantitative determination of sugars on filter paper chromatograms by direct photometry. Anal. Chem. 23: 1146-1149. 1951.

(37) Miller, Byron S., and John A. Johnson.
High levels of alpha-amylase in baking. II. Proteolysis in straight and sponge doughs. Cereal Chem. 25: 178-190. 1948.

(38) Miller, Byron S., John A. Johnson, and Donald L. Palmer.
A comparison of cereal, fungal, and bacterial alpha-amylases as supplements for breadmaking. Food Tech. 7:38-42. 1953.

(39) Miller, Sidney E., a nd Marjorie H. Iwen.
Reserve carbohydrates of leguminous seeds. Jour. Amer. Chem. Soc. 69: 2621-2625. 1947.

(40) Nussenbaum, Siegfried, and W. Z. Hassid.
Estimation of molecular weight of starch polysaccharides - determination of their reducing end-groups. Anal. Chem. 24:501-503. 1952.

(41) Pazur , J. H., and R. M. Sandstedt.
Identification of the reducing sugars in amylolysates of starch and starch-oligosaccharides. Presented at Tri-Section Meeting- Amer. Assoc. Cereal Chemists, Manhattan, Kansas, October, 1953.

(42) Philipp, Howard J.
New Dynamic method for osmotic pressure measurements. Jour. Polymer Sci. 6:371-380. 1951.

(43) Pyler, E. J.
Baking science and technology. Chicago: Siebel. Volumes I and II. 803 p. 1952.

(44) Rankin, John C., and Allene Jeanes.
Evaluation of the periodate-oxidation method for structural analysis of dextrans. Presented at the meeting of the American Chemical Society, Kansas City, Missouri, March, 1954.

(45) Sandstedt, R. M., M. J. Blish, D. K. Mecham, and C. E. Bode.
Identification and measurement of factors governing diastasis in wheat flour. Cereal Chem. 14: 17-34. 1937.

(46) Sandstedt, R. M., Eric Kneen, and M. J. Blish.
A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. 16: 712-723. 1939.

(47) Sarker, P. B.
Decomposition of formic acid by periodate. Nature. 168: 122-123. 1951.

(48) Schoch, Thomas J., and Dexter French.
Studies on bread staling. I. The role of starch. Cereal Chem. 24: 231-249. 1947.

(49) Schultz, A. S., F. D. Schoonover, R. A. Fisher, and S. S. Jackel. Retardation of crumb starch staling in commercial bread by bacterial alpha-amylase. Cereal Chem. 29:200-211. 1952.

(50) Somogyi, M.
A new reagent for the determination of sugars. Jour. Biol. Chem. 160: 61-68. 1945.

(51) Strain, Harold H., and George M. Murphy.
Chromatography and analogous differential migration methods. Anal. Chem. 24:50-60. 1952.

(52) Williams, Kenneth T., Arthur Bevenue, and Barbara Washauer.
A study of the use of ion-exchange resins for the removal of non-sugar reducing substances in the analysis of fresh and dehydrated vegetables for reducing sugars. Jour. Official Agri. Chemists. 30: 986-994. 1950.

APPENDIX

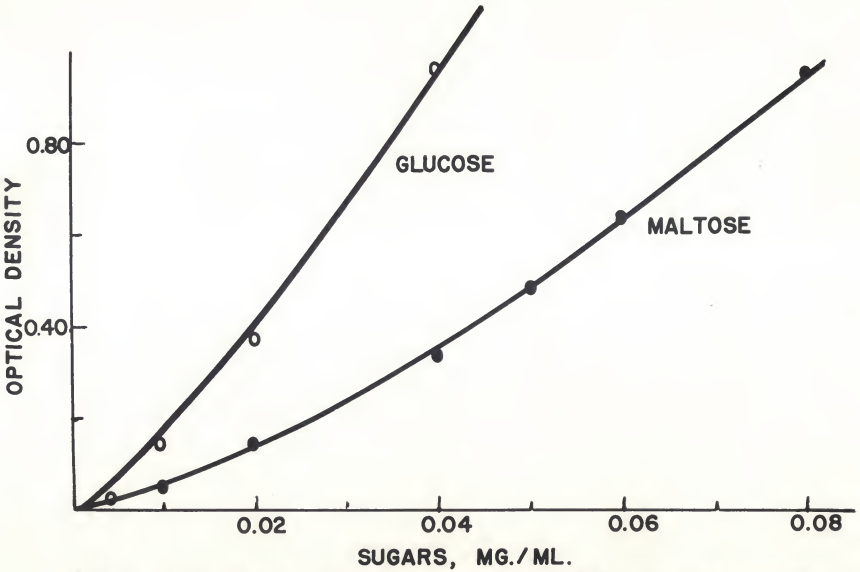

Fig. 3. Standard curve for determination of reducing groups by the colorimetric ferricyanide method.

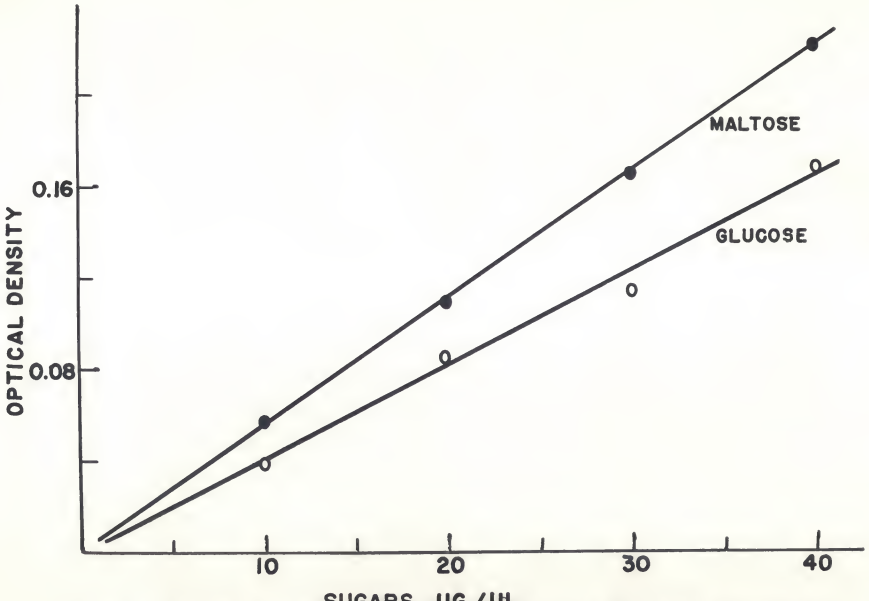

Fig. 4. Standard curve for determination of maltose and glucose by chromatographic analysis.

Table 3. Effect of alpha-amylase type and concentration on amounts of sugar in bread crumb.

Enzyme	Alpha-amylase SKB units (46)	Glucose	Maltose	Sum
		mg/gm bread crumb		
Control	None	4.0	1.1	5.1
Malted wheat flour	140	6.1	4.0	10.1
Malted wheat flour	560	8.1	6.5	14.6
Malted wheat flour	1120	11.1	7.0	18.1
Control	None	4.0	1.1	5.1
Fungal	140	6.4	1.9	8.3
Fungal	560	8.4	1.9	10.3
Fungal	1120	10.4	1.9	12.3
Control	None	4.0	1.1	5.1
Bacterial	7	---	---	---
Bacterial	35	5.9	1.0	6.9
Bacterial	140	11.2	4.9	16.1

CHEMICAL STUDIES ON THE SOLUBLE STARCH FRACTION IN BREAD

by

HORST BECK

B. S., University of Porto Alegre, Brazil, 1944

AN ABSTRACT OF A THESIS

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The purpose of this study was to characterize the carbohydrate material of bread which is modified during baking by different types and concentrations of alpha-amylase supplements and to relate these data to the characteristics of the bread crumb. The bread characteristics studied included concentration of maltose and glucose, and quantity and average chain length of the soluble dextrins. Fungal, cereal, and bacterial alpha-amylases were used to modify the carbohydrates in bread.

Breads were baked with three different levels of each of the alpha-amylase types. In addition, a control without alpha-amylase was included. One hour after baking, the sugars were extracted from portions of the bread crumb by refluxing with ethyl alcohol. The extract was separated from the insoluble material by centrifuging. After concentration of the sugars by removal of the alcohol, followed by de-ionization, the sugars were resolved on paper chromatograms. The carbohydrate residue of the alcohol extraction was extracted with water at 30° C., centrifuged, and used for the determination of the amount of soluble dextrins and their average chain length. Three methods were used to determine chain length of the water soluble dextrins. These included: periodate oxidation, ferricyanide reduction, and colorimetric ferricyanide methods. The quantity of water soluble dextrins was determined gravimetrically after precipitation of the dextrins with 80 per cent ethyl alcohol.

The loaf volume increased with increasing concentrations of alpha-amylase. The increases were greater with cereal and fungal than with bacterial amylase. The bread grain and texture scores

were improved by small amounts of alpha-amylase but high concentrations were detrimental. A high concentration of bacterial amylase was particularly detrimental since it tended to produce a sticky and gummy bread crumb. Sticky bread crumb was attributed to the severe modification of the starch and formation of the dextrins.

Increasing amounts of alpha-amylase caused an increase in the amount of extractable dextrins. The increase was small with fungal, larger with cereal, and largest with bacterial amylase. The average chain length of the soluble dextrins also increased with increasing concentrations of amylase. The ferricyanide, and the colorimetric ferricyanide method gave results which were comparable with each other but which did not agree with those obtained by the periodate method. The reasons for this are not clear. The residual glucose content was higher in bread crumb produced with bacterial than in that produced with cereal or fungal alpha-amylase. The fungal amylase increased the maltose content in the bread crumb less than did bacterial or cereal amylase. The differences in effects produced by three types of alpha-amylase primarily are attributed to differences in thermostability.